Chapter 5

Location of bladder and urethral sphincter motoneurons in the male guinea pig *(cavia porcellus)*

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Abstract

Although the guinea pig is used widely in experimental medical research, including in studies on micturition control, the spinal origin of preganglionic parasympathetic bladder and somatic external urethral sphincter motoneurons is not known. In the male guinea pig using wheat germ agglutinin-conjugated horseradish peroxidase and dextran Alexa Fluor 488/568 tracers, preganglionic parasympathetic bladder motoneurons were observed in the ventrolateral part of the intermediolateral cell group of the first sacral segment. The external urethral sphincter motoneurons were found to be located in the ventral horn of the first sacral segment, in a cell group corresponding with the nucleus of Onuf in cat and human.

Introduction

In studies on micturition, the guinea pig is used widely as an experimental animal since the anatomy of its lower urinary tract and its urodynamic profile of micturition are, in contrast to rat, similar to human (Van Asselt et al., 1995; Neuhaus et al., 2001). While the motoneuronal location of part of its perineal musculature has been described (Freeman and Breedlove, 1995), the location of motoneurons of bladder detrusor and external urethral sphincter (EUS) muscles, which are crucial for micturition and urine storage, has not been investigated yet. Firing of preganglionic parasympathetic bladder motoneurons results in contractions necessary to empty the bladder. These preganglionic neurons innervate, via the pelvic nerve, the postganglionic neurons of the detrusor muscle in the bladder wall. Bladder preganglionic motoneurons are located in the intermediolateral cell column (IML) of the lumbosacral spinal cord, in rat at L6-S1 (Nadelhaft and Booth, 1984), cat (Morgan et al., 1979), dog (Petras and Cummings, 1978) and monkey (Nadelhaft et al., 1983) at S1-S3. In cat, these motoneurons are located in the ventrolateral IML, while preganglionic parasympathetic motoneurons innervating

the colon are located dorsomedially (de Groat et al., 1979). The EUS muscle is the main closure muscle of the bladder and urethra. In the guinea pig, as in man, the EUS is a thin ring of striated muscle surrounding the urethra from the bladder base to the bulbourethral glands (Neuhaus and others, 2001). In mammalian species such as hamster (Gerrits et al., 1997), dog (Petras and Cummings, 1978; Kuzuhara et al., 1980), cat (Sato et al., 1978; Kuzuhara and others, 1980) and monkey (Nakagawa, 1980; Roppolo et al., 1985) EUS motoneurons are located in the so-called nucleus of Onuf (ON) in caudal S1 and in S2. In these animals ON is located in the ventrolateral aspect of the ventral horn and sends its axons through the pudendal nerve. In the rat, however, ON consists of two separate cell groups, a dorsomedial group and a ventrolateral group. The EUS motoneurons are located in the ventrolateral group at L5-L6 while anal sphincter motoneurons are located dorsomedially (Schroder, 1980; McKenna and Nadelhaft, 1986). The aim of the present study was to identify the spinal location of preganglionic parasympathetic bladder motoneurons and somatic EUS motoneurons in the guinea pig and to compare results obtained with other species.

Methods

Surgical procedures, pre- and postoperative care and handling and housing of the animals were approved by the Ethical Committee of the Faculty of Medical Sciences of the University of Groningen, The Netherlands. A total of six adult male guinea pigs (Cavia porcellus, Dunkin-Hartley, Harlan Nederland BV) weighing 600–1000 g were used. Animals were anesthetized with a combination of xylazine (5 mg/kg i.m.) and ketamine (40 mg/kg i.m.). In three animals the bladder was exposed after ventral

abdominal incision. Using a Hamilton syringe, 80 nl of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) (Sigma, 2.5% in saline, multiple injections of 10 ml per injection site) was injected throughout the entire surface of the detrusor muscle cranial to the bladder neck. WGA-HRP was used as a retrograde tracer

in these bladder cases because earlier nonpublished experiments revealed that dextran Alexa fluorescent tracers injected in the bladder did not produce labeling in the spinal cord. It is possible that dextran Alexa is not taken up by the bladder wall ganglia. In three other animals, using a Hamilton syringe, 15–20 nl of the retrograde fluorescent tracer Alexa Fluor dextran 488 or 568 (2% solution in distilled water, Molecular Probes Europe BV) was injected in the proximal urethra, about 5 mm distal to the bladder neck. This part of the urethra contains the EUS (Neuhaus et al., 2001) and is located under the pubic symphysis. In order to expose the EUS the bladder was gently pulled in a cranial direction. In the WGA-HRP

bladder experiments, following recovery from surgery and after a survival time of 48 h, the animals were deeply anesthetized with pentobarbital. Subsequently, the animals were perfused transcardially with 800 ml of saline at 37°C followed by 800 ml of 0.1 M phosphate buffered fixative containing 2% glutaraldehyde, 1% paraformaldehyde and 4% sucrose. Spinal cords were removed, postfixed for 1 h in the same fixative and cryoprotected by overnight storage in 0.1 M phosphate buffered 25% sucrose. The next day the spinal cord was frozen in an isopentane bath (-55°C) and cut in transverse sections of 40 mm. Every second section was processed serially using the tetramethylbenzidine (TMB) procedure. In the EUS experiments in which the fluorescent Alexa dextran tracers were used, following recovery from surgery and a survival time of 120 h, animals were deeply anesthetized with pentobarbital and perfused transcardially with 0.1 M phosphate buffered fixative of 4% paraformaldehyde. Spinal cords were removed, postfixed for 1h in the same fixative and cryoprotected by overnight storage in 0.1 M phosphate buffered 25% sucrose. The next day the spinal cord was frozen in an isopentane bath (-55°C) and cut in transverse sections of 40 mm. The distribution of retrogradely labeled neurons was examined with a Zeiss Axioplan microscope with polarized darkfield illumination in the WGA-HRP cases. In the cases with dextran Alexa Fluor 488 and 568 tracers, a Zeiss fluorescence filterblock was used with filterset 09 (excitation 450–490; emission 515) for dextran Alexa Fluor 488, and filterset 14 (excitation 510–560; emission 590) for dextran Alexa Fluor 568. All labeled motoneurons in every second section of the lumbosacral spinal

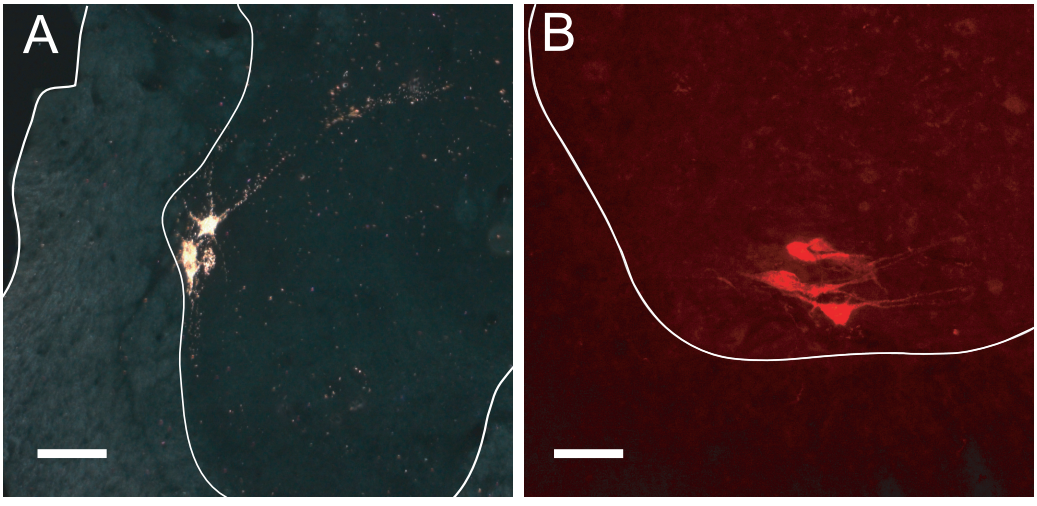

Figure 1. Photomicrographs of S1 sections. A: Retrogradely WGA-HRP labeled bladder motoneurons in the IML, using polarized darkfield illumination. Bar represents 200µm. B: Retrogradely Alexa Fluor 488 labeled EUS motoneurons in ON. Bar represents 100µm.

cord were counted and schematic drawings were made using a drawing tube. In all cases, photomicrographs of retrogradely labeled motoneurons were taken using a Leica DM-500 digital camera connected to the microscope.

Results

All bladder injections resulted in retrogradely labeled neurons bilaterally in the IML, but only in its ventrolateral part (figure 1). The labeled neurons were located mainly in S1 but in two of the three EUS cases very few labeled neurons were found in the most caudal L6 and the most rostral S2 segments (figure 2; table 1). In all EUS injected cases, a group of retrogradely labeled neurons was observed in the ventrolateral part of the ventral horn of the sacral spinal cord (figure 1). The group was located in S1 (figure 2), but in two of the three cases a few labeled motoneurons were found in the most caudal L6 and most rostral S2 segments (table 1). Since the EUS is a midline structure it was injected bilaterally, resulting inretrogradely labeled motoneurons on both sides of the cord.

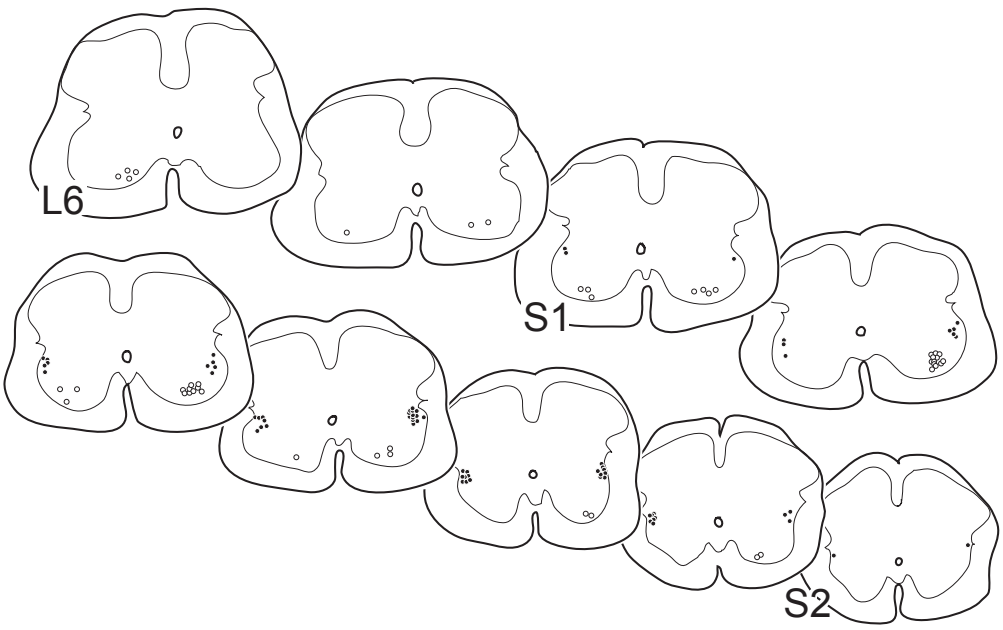

Figure 2. Schematic compilation of two cases. The location of WGA-HRP retrogradely labeled bladder motoneurons in case GP9 is indicated with (●). The dextran Alexa Fluor 488 retrogradely labeled EUS motoneurons in case GP2 are indicated with (○). Each drawing represents six sections.

			segments			
case	**injection**	**tracer**	**L6**	**S1**	**S2**	**total**
GP2	Bladder	WGA-HRP	1	35	2	38
GP6	Bladder	WGA-HRP	1	30	8	39
GP9	Bladder	WGA-HRP	0	70	0	70
GP2	EUS	Alexa 488	0	19	0	19
GP7	EUS	Alexa 488	7	37	2	46
GP8	EUS	Alexa 568	3	43	4	50

Table 1. Number of retrogradely labeled neurons found in the L6-S2 segments in each case

Discussion

These results demonstrate that the ventral horn location of motoneurons that innervate the bladder in the guinea pig is similar to other mammalian species. The motoneurons were found in S1, while in rat they are located at L6-S1 (Nadelhaft and Booth, 1984), and in cat (Morgan and others, 1979), dog (Petras and Cummings, 1978) and monkey (Nadelhaft and others, 1983) they are located at S1-S3. The location of the EUS motoneurons is, as in other species, in a region of the ventral horn corresponding with ON in cat and human. The EUS motoneurons were found rostrocaudally in S1 while in rat they are located at L5-L6 (Schroder, 1980; McKenna and Nadelhaft, 1986) and in cat at S1-S2 (Sato and others, 1978; Kuzuhara and others, 1980). The number of observed motoneurons was limited but the goal of this investigation was to establish the location of the bladder and EUS motoneurons, not to determine the precise number. In none of the cases did the tracer injections involve the entire bladder or EUS, which means that the number of observed retrogradely labeled motoneurons is always lower than the total number of motoneurons innervating the muscle. These findings serve as a basis for further studies on the neuronal control of micturition in the guinea pig. The present results correspond with the view that micturition control in guinea pig is similarly organized as in cat. In the cat, two pontine cell groups, the so-called M- and L-regions, strongly control micturition by way of projections to the sacral spinal cord (Holstege et al., 1986). The next step is to determine whether such pontine regions exist in the guinea pig also.

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